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'Spodoptera frugiperda' Nuclear Polyhedrosis Virus Genome: Physical Maps for Restriction Endonucleases BamHI and HINDIII

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Spodoptera frugiperda Nuclear Polyhedrosis Virus Genome: Physical Maps for Restriction Endonucleases *Bam*HI and *Hin*dIII

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The physical map for the genome of *Spodoptera frugiperda* nuclear polyhedrosis virus was constructed for restriction endonucleases *Bam*HI and *Hin*dIII. The ordering of the restriction fragments was accomplished by cross-blot hybridization of *Bam*HI, *Hin*dIII, and *Eco*RI fragments. The alignment of the *Hin*dIII fragments within the *Bam*HI map was achieved by double digestion with the two restriction endonucleases followed by cross-blot hybridization. The results showed that the viral genome consisted of mainly unique sequences. In addition, the circular nature of the viral genome was reaffirmed.

Spodoptera frugiperda nuclear polyhedrosis virus (SF NPV) is a member of the baculovirus group with a double-stranded, circular, supercoiled DNA genome (16). The molecular weight of the DNA molecule has been determined to be about 80×10^6 by electron microscopy and restriction endonuclease analysis in this laboratory (manuscript submitted), in reasonable agreement with previous reports by other investigators (1, 2, 4, 7, 8, 16).

Because of its relatively large size and complexity, the construction of a restriction map of the viral genome should greatly facilitate the study of its molecular biology. It would allow investigators to study virus recombinants and map the crossover sites (14, 17). The comparison of different virus strains by restriction endonuclease analysis (9, 13) is more meaningful when it is possible to locate the regions showing the greatest genetic variation between virus strains. Even more important, it is essential for the study of gene transcription and regulation in both permissive and non-permissive cells. In this paper, a physical map of the SF NPV genome was constructed with the restriction endonucleases *Bam*HI and *Hin*dIII.

MATERIALS AND METHODS

Virus purification. The partial purification of polyhedra of the Ohio strain of SF NPV was performed at the Southern Grain Insects Laboratory from the lysate of virus-infected Fall army worm larvae in Tifton, Ga. Subsequently, this suspension of polyhedra was washed by repeated centrifugation in a Sorvall RC-5 refrigerated centrifuge at 5,000 rpm for 5 min and resuspension in distilled water until the supernatant fluid was clear. After the last centrifugation, the polyhedra were suspended in a freshly prepared solution of 0.1 M Na_2CO_3 for 15 to 20 min at room temperature and centrifuged for 5 min at 5,000 rpm. This procedure was repeated two to three times until the alkaline degradation of the polyhedra was complete. The supernatant from each centrifugation was pooled, layered gently onto 20 to 60% (wt/wt) sucrose gradients made in TBS (0.15 M NaCl–0.05 M Tris–hydrochloride, pH 7.4) and centrifuged in an SW-27 rotor for 75 min at 25,000 rpm and 4°C. The multiple virus bands in each gradient were collected through the bottom of each tube, dialyzed against TBS overnight, and stored at 4°C. If a more concentrated virus preparation is desired, the virions may be pelleted by centrifugation and resuspended in an appropriate buffer.

Extraction of viral DNA. A concentrated suspension of gradient-purified virions was digested at 55°C for 1.5 h in a proteinase K digestion mixture (1% sodium dodecyl sulfate–5 mM $CaCl_2$–1 mM EDTA–100 μg of proteinase K per ml). When pronase (1 mg/ml) was substituted for proteinase K, the digestion was carried out at 37°C.

The digested mixture was carefully layered onto preformed 10 to 30% (wt/vol) sucrose gradients in TBS containing 1 mM EDTA and centrifuged in SW-27 rotors at 18,000 rpm for 18 h in a Beckman L3-40 ultracentrifuge. Fractions (1 ml) were collected through the top by pumping a 60% sucrose solution through the bottom of the tube. The DNA-containing fractions as determined by UV absorption were pooled, dialyzed against 0.05 M Tris–hydrochloride (pH 7.4)–1 mM EDTA buffer overnight and stored at 4°C.

Alternatively, a modification of the method of Radloff et al. (10) was used. The proteinase K-digested mixture was diluted with 0.01 M Tris-hydrochloride (pH 8.0)–0.01 M EDTA buffer containing ethidium bromide at a concentration of 200 μg/ml. Cesium chloride was added to the solution until the density was 1.59 g/ml. Centrifugation was carried out in a VI-

50 rotor at 36,000 rpm for 24 h in a Beckman ultracentrifuge. The DNA-containing fractions were visualized by their fluorescence in UV light and collected. The ethidium bromide was removed by extraction with isoamyl alcohol, and the DNA fractions were dialyzed against 0.05 M Tris-hydrochloride (pH 7.4)–0.001 M EDTA buffer overnight and stored at 4°C or further purified by sedimentation through a sucrose gradient as previously described.

If intact DNA molecules were not required, the DNA solution could be extracted with phenol, and the DNA could be precipitated with cold ethanol at −20°C and redissolved in an appropriate buffer.

Restriction enzyme digestion of viral DNA. Purified viral DNA was digested with *Eco*RI, *Bam*HI, or *Hin*dIII in a solution containing 0.05 M Tris-hydrochloride (pH 7.4)–0.01 M $MgCl_2$–0.10 M NaCl–0.006 M β-mercaptoethanol. Incubations were generally for 3 h at 37°C, and sufficient enzyme was added for complete digestion within this period. When partial digestion of the viral DNA became necessary for mapping purposes, the same amount of restriction enzyme was added, but incubation was carried out at 10°C for between 10 and 30 min. The reaction was stopped by adding EDTA to a final concentration of 10 mM. The restriction enzyme was then heat inactivated at 70°C for 20 min.

The restriction enzyme *Eco*RI was purified from *Escherichia coli* strain Ry13 (3), *Hin*dIII was purchased from Bethesda Research Laboratories, Inc., Rockville, Md., and *Bam*HI was purified from *Bacillus amyloliquefaciens* H (19; L. A. Smith and J. G. Chirikjian, Fed. Proc. **36:** 908, 1977).

In vitro labeling of DNA. For critical determinations of the molecular weights and stoichiometry of DNA fragments by electrophoresis in agarose gels, the DNA restriction fragments were end labeled in vitro with avian myeloblastosis virus reverse transcriptase obtained from G. E. Houts of the Life Sciences Institute, St. Petersburg, Fla. Approximately 10 μCi of [α-^{32}P]dATP (450 Ci/mmol; ICN Pharmaceuticals) were lyophilized in a small tube and redissolved in a reaction mixture containing 20 to 25 μg of DNA restriction fragments per ml, 100 mM NaCl, 50 mM Tris-hydrochloride (pH 7.4), 6 mM β-mercaptoethanol, 10 mM $MgCl_2$, 1 mM $MnCl_2$, 0.1 mM dCTP, 0.1 mM dGTP, and 0.1 mM dTTP. Reverse transcriptase (15 U) was added, and incubation was carried out at 4°C for 20 to 30 min. The reaction was stopped by heat inactivating the enzyme in a 70°C water bath for 20 min. The mixture was then treated with proteinase K (50 μg/ml) for 30 min at 37°C before gel electrophoresis.

For cross-blot hybridization experiments, labeling was done with DNA polymerase I instead of reverse transcriptase. The procedure, a modification of the method of Rigby et al. (12), was essentially the same as that described in the preceding paragraph, except that 5 μl of DNA polymerase I was used, the $MnCl_2$ was not needed, and incubation was carried out at 14°C for 30 min. It was found that this procedure can be adopted for end labeling DNA restriction fragments as well. The DNA polymerase I used in these experiments was purified by the method of Richardson et al. (11) as modified by Jovin et al. (6).

Agarose gel electrophoresis of DNA fragments. The DNA restriction fragments were fractionated by electrophoresis through 0.7% agarose (SeaKem) gels. Agarose was dissolved in E buffer (0.04 M Tris-hydrochloride–0.02 M sodium acetate–1 mM EDTA, pH 7.2) and allowed to solidify on a glass plate to form a horizontal slab gel (6 by 200 by 250 mm). For cross-blot hybridization epxeriments, slightly wider gels (6 by 250 by 250 mm) were used. Sample slots of suitable sizes were formed by inserting a plexiglas comb at one end of the gel before gel formation was complete. DNA samples were loaded into the slots with tracer amounts of a bromophenol blue solution made up in E buffer with 30% glycerol.

The DNA was electrophoresed at a constant voltage of 100 V at 25°C for about 18 h until the bromophenol blue marker reached the end of the gel.

Visualization of DNA fragments. When unlabeled DNA samples were electrophoresed, the gel was stained with ethidium bromide (20 μg/ml in E buffer) for 1 h, and the DNA fragments could be visualized under UV light and photographed with a Polaroid MP-4 camera with an orange filter under the lens.

In the case of labeled DNA samples, the gel was dehydrated by vacuum on a gel dryer and exposed to Kodak X-Omat R X-ray film for 24 to 48 h. The positions of the DNA fragments were thus recorded on the developed film. For molecular weight measurements, lambda DNA digested with *Eco*RI, *Bam*HI, or *Hin*dIII was used for molecular weight markers. The migration distances of the DNA fragments as recorded on film were determined by a Hewlett Packard 9825A calculator and digitizer programmed to calculate the DNA fragment sizes in kilobases when suitable molecular weight markers were provided. Densitometer tracings of the autoradiographs were done with a Joyce Loebl microdensitometer. The molar ratios of the DNA fragments, being proportional to the areas under the peaks, were then determined by cutting out and weighing the corresponding peaks on these tracings.

Cross-blot hybridization. To detect homology between different fragments, the Hutchison cross-blot hybridization technique (C. Hutchison, personal communication) was used. A "cold" restriction digest was run across the entire width of a square slab gel (6 by 250 by 250 mm) using 10 to 15 μg of viral DNA per gel. The positions of the DNA bands could be photographed under UV light after staining with ethidium bromide. The DNA in the gel was then depurinated partially with 0.25 M HCl, cleaved and denatured with 0.5 M NaOH–1 M NaCl, and neutralized in 1 M Tris-hydrochloride (pH 7.4)–1.5 M NaCl to improve the efficiency of transfer of large DNA fragments onto a nitrocellulose sheet (18). The actual transfer was done in 6× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) at room temperature for 8 h by the procedure of Southern (15) with slight modifications. The nitrocellulose sheet (Bethesda Research Laboratories) was then air dried briefly and baked in vacuum at 80°C for 3 h.

A ^{32}P-labeled restriction digest was run and prepared for transfer as described above. The nitrocellulose sheet with the immobilized DNA was placed on top of the radioactive gel such that each cold band bound to the nitrocellulose sheet intersected each

"hot" band in the gel at right angles. The hot band patterns were then transferred and directly hybridized at right angles to the unlabeled DNA bands on the nitrocellulose sheet in hybridization buffer (6× SSC–0.1% sodium dodecyl sulfate) at 65°C for 8 h in a humidified oven. All components of the system except the gel were preheated to 65°C before the transfer and hybridization step. Finally, the nitrocellulose sheet was washed as described by Jeffreys and Flavell (5), blotted dry, wrapped in a protective plastic freezer bag, and exposed to Kodak X-Omat R X-ray film for 3 days. A Du Pont Lightning-Plus intensifier screen was often used to shorten the exposure time. After development, spots of developed silver grains should mark the intersection points of DNA fragments sharing common sequences.

RESULTS

Restriction enzyme cleavage patterns. The SF NPV genome was cleaved into 8, 15, and 25 fragments by the restriction enzymes *Bam*HI, *Hin*dIII, and *Eco*RI, respectively (Fig. 1). The estimated molecular weights of the restriction fragments (see Table 6) have already been reported (manuscript submitted). As described in a later section, slight corrections to the molecular weights of *Bam*HI fragments were made. It is seen that there are four sets of comigrating fragments in the *Eco*RI digest, one in the *Hin*dIII digest, and none in the *Bam*HI digest. Thus, it was decided that the construction of restriction maps for *Hin*dIII and *Bam*HI should be attempted initially because they would allow less ambiguous assignment of transcription patterns and regions of genetic variation between virus strains by using the Southern blot hybridization technique.

Construction of a *Bam*HI restriction map by cross-blot hybridization. The first step in the mapping procedure was to determine whether there were repeat sequences within the viral genome which could complicate the interpretation of hybridization data. This was accomplished by the cross-blot hybridization of cold *Bam*HI fragments to ^{32}P-labeled *Bam*HI fragments (Fig. 2). Spots were found along the diagonal only. Thus, there were probably no long repeat sequences present in the viral genome.

Partial digestion of SF NPV DNA should produce DNA fragments consisting of two or more "complete" restriction fragments linked together. By hybridizing ^{32}P-labeled *Bam*HI fragments to unlabeled, partially digested *Bam*HI fragments immobilized on a nitrocellulose sheet, the linkages between different *Bam*HI restriction fragments could be deduced (Fig. 3 and Table 1). The deduction of the single linkages from fragments 3, 4, 5, and 6 are quite obvious. Fragment 2 has four spots, indicating sequence homologies with *Bam*HI fragments B,

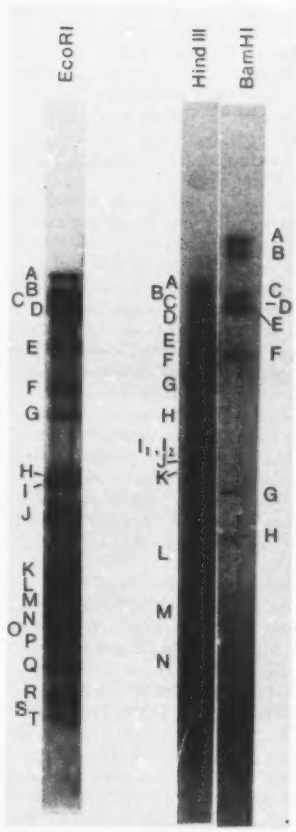

FIG. 1. *Cleavage patterns of SF NPV DNA by the restriction endonucleases BamHI, HindIII, and EcoRI. The restriction fragments were labeled with [α-^{32}P]dATP in the presence of DNA polymerase I. The partial fragment between fragments D and E in the EcoRI digest was the result of slightly incomplete digestion.*

E, F, and G. One of the two comigrating fragments must be fragment B, because one spot is on the diagonal where the labeled and unlabeled B fragments should intersect. Thus, the three *Bam*HI fragments E, F, and G must be linked together to form the other comigrating fragment. Since fragment E is directly linked to both fragments F and G, the three fragments must be linked in the manner indicated in Table 1, i.e., F-E-G. To sum up, the data in Table 1 demonstrate linkages between five *Bam*HI fragments, and the deduced ordering is H-C-F-E-G.

To complete the ordering of *Bam*HI restriction fragments, ^{32}P-labeled *Hin*dIII fragments were hybridized to unlabeled *Bam*HI fragments in another cross-blot hybridization experiment

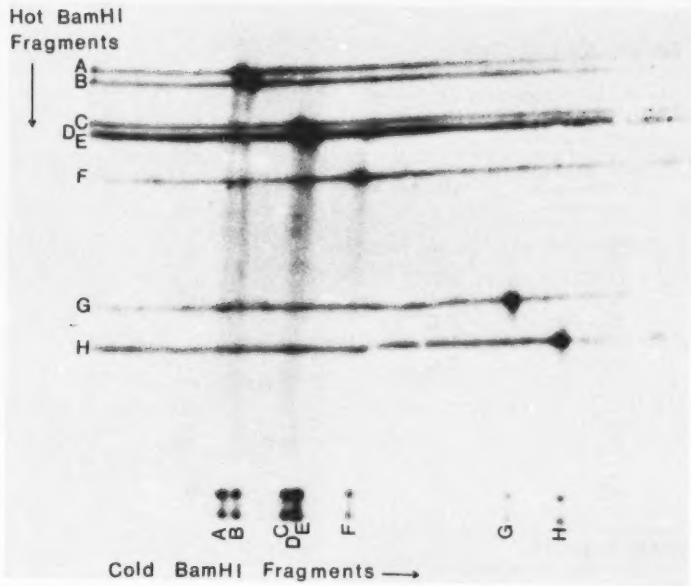

FIG. 2. *Autoradiograph of the patterns resulting from cross-blot hybridization between ^{32}P-labeled and cold BamHI fragments of SF NPV DNA. A ^{32}P-labeled BamHI digest was run side by side with the cold BamHI digest and transferred to the nitrocellulose sheet. The identification of the cold BamHI fragments was thus facilitated by noting the position of the corresponding transferred ^{32}P-labeled fragment.*

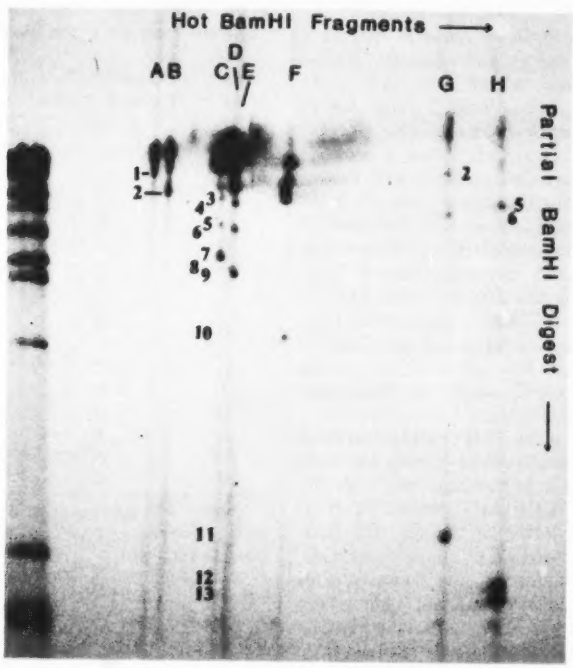

FIG. 3. *Autoradiograph of the patterns resulting from the cross-blot hybridization between ^{32}P-labeled BamHI fragments and a cold partial BamHI digest of SF NPV DNA. The ^{32}P-labeled fragments of the partial digest was run side by side with the cold fragments and transferred to the nitrocellulose sheet to facilitate the identification of the unlabeled fragments.*

TABLE 1. *Cross-blot hybridization of ^{32}P-labeled BamHI fragments to unlabeled BamHI partial digestion products*

Partial digestion products	*Bam*HI fragments with homologous sequences	Linkage groups
1	A	
2[a]	B	
	E, F, G	F-E-G
3	C, F	C-F
4	E, F	F-E
5	C, H	H-C
6	E, G	E-G
7	C	
8	D	
9	E	
10	F	
11	G	
12	H[b]	
13	H	

[a] Two comigrating fragments.

[b] Fragment 12 probably corresponds to *Bam*HI fragment H linked to a short fragment since fragment 13 is on the diagonal marking the intersection points of identical cold and ^{32}P-labeled *Bam*HI fragments. The short fragment was not observed in the 0.7% agarose gels because its small size allowed it to migrate ahead of the bromophenol blue marker.

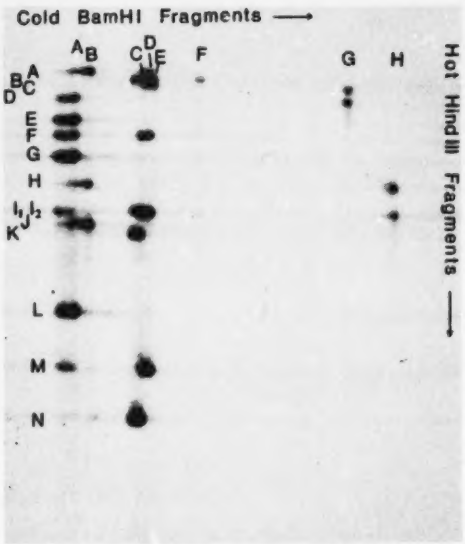

FIG. 4. *Autoradiograph of the patterns resulting from the cross-blot hybridization between ^{32}P-labeled HindIII fragments and cold BamHI fragments of SF NPV DNA. The smearing of the spots was due to the slight overloading of the sample slots during gel electrophoresis of the cold BamHI fragments.*

(Fig. 4 and Table 2). If a *Hin*dIII fragment shares sequence homology with only one *Bam*HI fragment as is the case with fragments E, G, I_1, J, K, L, M, and N, the *Hin*dIII fragment in question must be entirely within the *Bam*HI fragment involved. However, when a *Hin*dIII fragment shares sequence homology with two or more *Bam*HI fragments, these *Bam*HI fragments must be linked together with the *Bam*HI restriction sites at the linkage points lying within the *Hin*dIII fragment involved. This is illustrated in Table 2 by the *Hin*dIII fragments A, B, C, D, F, H, and I_2. Thus, with *Bam*HI fragment B linked to fragments H and D and *Bam*HI fragment A linked to fragments D and G, the ordering of the *Bam*HI restriction fragments could be completed (Fig. 5a).

Construction of a *Hin*dIII restriction map by cross-blot hybridization. Using the data presented in Table 2 in combination with the known ordering of the *Bam*HI restriction fragments, a partial ordering of the *Hin*dIII fragments could be deduced (Fig. 5b). Where uncertainties in ordering existed, the fragments involved were included in one block. This is true for *Hin*dIII fragments E, G, and L, which lie totally within *Bam*HI fragment A, *Hin*dIII fragments I_1 and M, which lie within *Bam*HI fragment D, and *Hin*dIII fragments K and N, which lie within *Bam*HI fragment C.

To clear up these ambiguities, ^{32}P-labeled

TABLE 2. *Cross-blot hybridization of ^{32}P-labeled HindIII fragments to unlabeled BamHI fragments*

*Hin*dIII fragment	*Bam*HI fragments with homologous sequences	Linkage groups for *Bam*HI
A	B, D	D-B
B	C, E, F	C-F-E
C	E, G	E-G
D	A, G	G-A
E	A	
F	A, D	A-D
G	A	
H	B, H	B-H
I_1	A[a], C, D, H	D
I_2		H-C
J	B	
K	C	
L	A	
M	A[a], D	
N	C	

[a] These two spots suggest a possible sequence homology between parts of *Bam*HI fragment D and *Bam*HI fragment A. The relevant sequences within *Bam*HI fragment D are located near the junction between *Hin*dIII fragments M and I_1. The sequence within *Bam*HI fragment A is located within *Hin*dIII fragment L (Fig. 8 and Table 5).

*Eco*RI fragments were hybridized to unlabeled *Hin*dIII fragments in a cross-blot hybridization experiment (Fig. 6 and Table 3). The linkage

groups were deduced by the reasoning described above. In the assignment of linkages deduced from comigrating *Eco*RI fragments C_1 and C_2, it is seen that *Hind*III fragments D and E must be linked because fragments H and I_2 are known to be linked and located well away from both D and E. However, the assignments involving *Eco*RI comigrating fragments D_1, D_2, and D_3 are more speculative. Fortunately, the ordering of the *Hind*III fragments involved is already precisely known. With the additional information in Table 3, the *Hind*III restriction map can now be deduced. It is seen that *Hind*III fragment E is linked to both fragments D and L. Thus, it is easy to reason that the fragments D, E, F, G, and L are joined in the order D-E-L-G-F. Similarly, knowing that fragment I_1 is linked to both fragments A and M, one can deduce that the fragments A, F, I_1, and M are linked in the order F-M-I_1-A. Finally, with *Hind*III fragment K linked to fragment B, the fragments B, I_2, K and N can be unambiguously joined as I_2-N-K-B. The resulting restriction map for *Hind*III is shown in Fig. 5c.

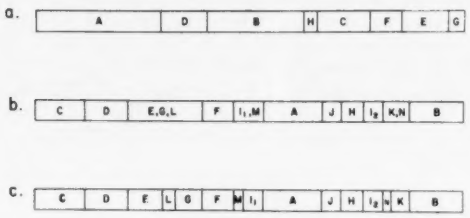

FIG. 5. *a, Physical map of SF NPV genome for Bam HI; b, partial physical map of SF NPV genome for HindIII; c, physical map of SF NPV genome for HindIII.*

Double digestion of SF NPV DNA by *Bam*HI and *Hind*III. The double digestion of SF NPV DNA by *Bam*HI and *Hind*III serves a threefold purpose. First, it provides an independent confirmation of the restriction map deduced from cross-blot hybridization. Second, it allows the precise alignment of *Hind*III restriction fragments within the *Bam*HI map or vice versa. Last, it gives better estimates of the molecular weights of large DNA fragments because these fragments are cleaved by the second en-

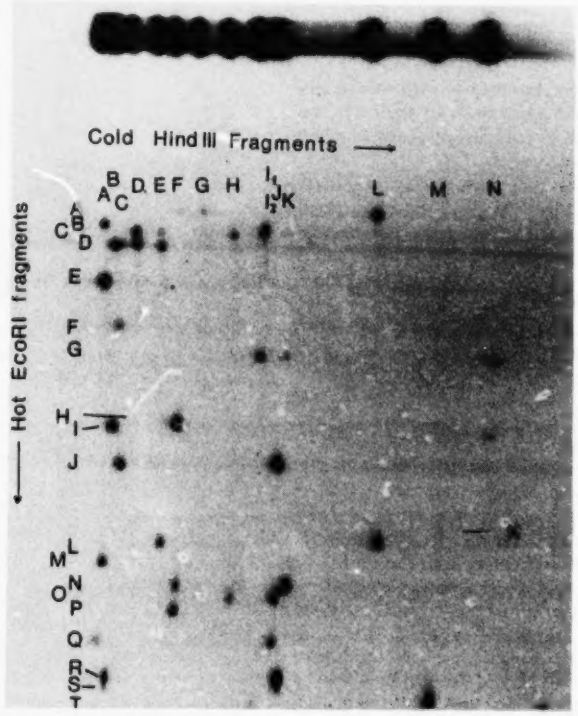

FIG. 6. *Autoradiograph of the patterns resulting from the cross-blot hybridization between ^{32}P-labeled EcoRI fragments and cold HindIII fragments of SF NPV DNA.*

TABLE 3. *Cross-blot hybridization of ^{32}P-labeled EcoRI fragments to unlabeled HindIII fragments*

*Eco*RI fragment	*Hin*dIII fragments with homologous sequences	Linkage groups for *Hin*dIII
A	F, G, L	L-G-F
B	A, I	I_1-A
C_1, C_2	D, E, H, I_1	D-E, H-I_2
D_1, D_2, D_3	B, C, D, E	B-C, C-D, E
E	A	
F	C	
G	I, K, N	I_2-N-K
H	F	
I	B	
J_1, J_2	C, J	
K	M	
L	E, L	E-L
M	A	
N_1, N_2	F, K	
O	H, J	J-H
P	F	
Q	A, J	A-J
R	B, K	K-B
S	B	
T	M, I	M-I_1

zyme into smaller fragments that are within the range of molecular weights provided by the markers. In our case, this is applicable to the *Bam*HI fragments A and B.

SF NPV DNA was first digested with *Hin*dIII and then with *Bam*HI, end labeled with [α-^{32}P]dATP, and electrophoresed in a 0.7% agarose gel. A complete *Hin*dIII digest was run at the same time so that the fragments that were cleaved by *Bam*HI could be identified. The molecular weights and stoichiometry of the restriction fragments were estimated as described above. The *Bam*HI and *Hin*dIII double-digest patterns are shown in Fig. 7. It is seen that the *Hin*dIII fragments B, C, D, F, H, and I_2 have disappeared. In addition, 13 new fragments are present. Referring to the restriction maps in Fig. 5, this is exactly what is expected, with *Hin*dIII fragments C, D, F, H, and I_2 each being cleaved once and fragment B cleaved twice. Fragment A should also be cleaved once. This is confirmed by its slight shift in position to one corresponding to a lower molecular weight. The cleaved-off piece of DNA is probably so short that it is running ahead of the bromophenol blue marker and has migrated out of the gel. The estimated molecular weights of these double-digestion products are presented in Table 4.

Alignment of *Hin*dIII fragments within the *Bam*HI map. To achieve the alignment of *Hin*dIII fragments within the *Bam*HI map, ^{32}P-labeled *Hin*dIII fragments were hybridized to unlabeled DNA fragments from a *Hin*dIII-*Bam*HI double digestion in a cross-blot hybridization experiment (Fig. 8). The ^{32}P-labeled *Hin*dIII fragments will hybridize to the double-digest fragments that share their sequence homology, thus allowing the identification of specific double-digest fragments that form the original *Hin*dIII restriction fragments (Table 5). The location of *Bam*HI sites within the *Hin*dIII fragments can then be calculated from the estimated molecular weights of the double-digestion fragments listed in Table 4. The alignment of *Hin*dIII fragments within the *Bam*HI map was thus accomplished. For example, the *Hin*dIII fragment D, made up of fragments 4 and 18 of the double digest, spans the *Bam*HI fragments

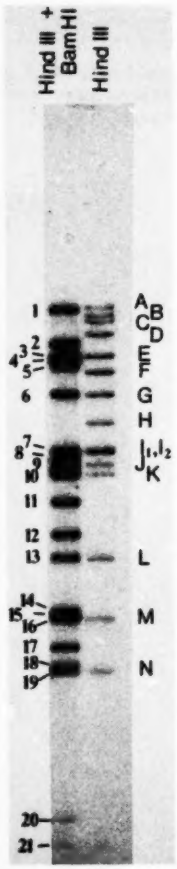

FIG. 7. *Cleavage patterns of SF NPV DNA by HindIII and (HindIII + BamHI). The restriction fragments were labeled with [α-^{32}P]dATP in the presence of DNA polymerase I.*

TABLE 4. *Molecular weights of HindIII-BamHI double-digest fragments*

Fragment	Mol wt (10^6)
1	11.1
2	7.8
3	7.0
4	6.7
5	6.4
6	5.3
7	3.9
8	3.8
9	3.7
10 (2 comigrating fragments)	3.5
11	3.1
12	2.7
13	2.4
14	1.9
15	1.8
16	1.8
17	1.6
18	1.5
19	1.4
20	0.72
21	0.59

A and G. Since fragment 4 is too long to fit into *Bam*HI fragment G, it must be within *Bam*HI fragment A, whereas fragment 18 lies within *Bam*HI fragment G. Similar deductions can be made, and the results are summarized in the third column of Table 5.

During the initial alignment, it was found that the previously determined molecular weights of *Bam*HI fragments A and B were too low. Thus, using the estimated molecular weights of the double-digestion fragments (Table 4), more accurate values of 24.1×10^6 and 18.5×10^6, respectively, were assigned to the *Bam*HI fragments A and B and used in the construction of the final restriction map of *Bam*HI. The molecular weights of the other *Bam*HI fragments were proportionally adjusted so that the size of the whole DNA molecule was again 82.5×10^6. The molecular weights of the *Hind*III and *Bam*HI restriction fragments used in the map are presented in Table 6. The restriction map of SF NPV DNA for *Bam*HI and *Hind*III is shown in Fig. 9.

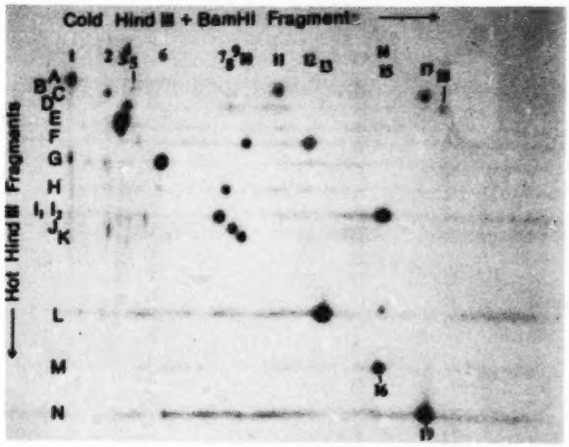

FIG. 8. *Autoradiograph of the patterns resulting from the cross-blot hybridization between ^{32}P-labeled HindIII fragments and cold HindIII and BamHI double-digested fragments of SF NPV DNA.*

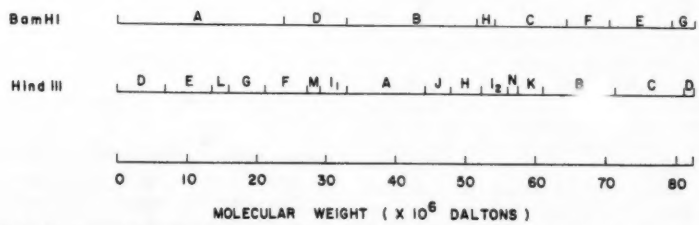

FIG. 9. *Physical maps of the SF NPV genome for the restriction endonucleases BamHI and HindIII. The BamHI fragment A is arbitrarily placed at the origin of the linear map for the sake of simplicity. The actual map should be circular.*

DISCUSSION

The restriction map of the SF NPV DNA genome is presented in Fig. 9 in linear rather than circular form for the sake of simplicity. However, the restriction enzyme mapping data confirm the circular nature of the DNA molecule. The SF NPV genome consists of mainly unique sequences. This is in agreement with the results from Kelly's reassociation kinetics experiments (7) and our previous partial denaturation mapping data (manuscript submitted). Our cross-blot hybridization data (Fig. 4 and 8, Tables 2 and 5) indicate that the only possible repeat sequences of any appreciable length may be those located in *Hin*dIII fragment L and those near the junction of *Hin*dIII fragments M and I_1. However, one cannot exclude the possibility of additional short repeat sequences within parts of the genome where no *Bam*HI, *Hin*dIII, or *Eco*RI restriction sites are present.

The restriction maps for *Bam*HI and *Hin*dIII sites should provide sufficient sectioning of the SF NPV genome to facilitate initial studies of

TABLE 5. *Sequence homology between the HindIII-BamHI double-digestion products and HindIII or BamHI restriction fragments*

Double-digest fragment	*Hin*dIII fragments with homologous sequences[a]	*Bam*HI fragments with homologous sequences[b]
1	A	B
2	C	E
3	E	A
4	D	A
5	B	F
6	G	A
7	H	B
8	I_1	D
9	J	B
10	F	D
(2 comigrating fragments)	K	C
11	B	C
12	F	A
13	L	A
14	I_2	H
15	I_2	C
16	L[c], M	A[c], D
17	C	G
18	D	G
19	N	C
20	B[d]	E
21	H[d]	H

[a] From cross-blot hybridization data.

[b] From restriction map alignment.

[c] The spot at the point of intersection of fragment 16 and fragment L suggests a possible sequence homology between *Hin*dIII fragments L and M (i.e., fragment 16 in the double digest). This is already indicated in Table 2.

[d] These are deduced by comparing the molecular weights of complete *Hin*dIII fragments and their double-digestion products as well as restriction map positions.

TABLE 6. *Molecular weights of the BamHI, HindIII, and EcoRI restriction fragments*

Fragment	Mol wt (10^6)
*Bam*HI	
A	24.1
B	18.5
C	10.2
D	9.1
E	8.8
F	6.0
G	3.2
H	2.6
*Hin*dIII	
A	11.3
B	10.2
C	9.6
D	8.4
E	6.9
F	6.1
G	5.3
H	4.4
I_1	3.9
I_2	3.9
J	3.6
K	3.5
L	2.4
M	1.8
N	1.4
*Eco*RI	
A	8.1
B	7.1
C_1	6.7
C_2	6.7
D_1	6.1
D_2	6.1
D_3	6.1
E	4.8
F	3.8
G	3.3
H	2.4
I	2.4
J_1	2.0
J_2	2.0
K	1.5
L	1.4
M	1.3
N_1	1.2
N_2	1.2
O	1.2
P	1.1
Q	1.0
R	0.90
S	0.87
T	0.82

transcription patterns and genetic variations between virus strains. The relatively precise locations of the *Bam*HI and *Hin*dIII restriction sites are confirmed by our double-digestion data where good placement of the sites for the second enzyme within the sites of the first enzyme is possible. If a specific region of the genome needs to be studied more closely, restriction mapping with a third or fourth enzyme such as *Eco*RI or *Xba*I may be attempted to provide an even smaller sectioning of the viral genome.

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